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Heterosexual transmission of human immunodeficiency virus type 1 subtype C in southern Brazil

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ABSTRACT

Background: Human immunodeficiency virus type 1 (HIV-1) subtype B predominates in Brazil, but in the southern region subtype C is the most frequent, followed by subtypes B, F1 and recombinant forms. In southern Brazil, these subtypes co-circulate in subjects with homogeneous demographic and clinical features, enabling a better understanding of the role of HIV-1 subtypes on the characteristics of infection.
Objectives: To evaluate the prevalence of different HIV-1 subtypes in subjects with recent diagnosis for HIV infection in the extreme south of Brazil, and to study their association with demographic, behavioral, clinical and laboratorial characteristics.
Study design: We have determined the genetic sequence of viral protease and reverse transcriptase (polymerase, connection and RNase H domains) isolated from studied subjects. Viral subtype was inferred by comparison with reference HIV sequences, and recombination was determined with Simplot analysis. The association of HIV-1 subtypes with studied characteristics was evaluated by chi-square, Fisher's exact, Student's *t* and Kruskal–Wallis tests.
Results: Two hundred and forty-five HIV isolates were molecularly characterized, and the association with variables was studied for 233 (95.1%) patients. Of those, 46.8% followed AIDS defining criteria. HIV-1C was responsible for 56.3% of infections, and was associated with heterosexual transmission ($p = 0.001$) and with higher CD4$^+$ T-cell counts ($p = 0.02$).
Conclusions: The molecular epidemiology of HIV-1 in the southernmost Brazil is currently steady with predominance of HIV-1C. This is the first study showing a robust association of the infection by this subtype and heterosexual transmission in the state of Rio Grande do Sul, Brazil.

1. Background

The global distribution of subtypes and circulating recombinant forms of human immunodeficiency virus type 1 (HIV-1) group M reflects the complexity of its molecular epidemiology. HIV-1 subtype C (HIV-1C) is identified in over 50% of worldwide infections, and greatly represented in countries with the largest prevalence of infection, such as those of southern Africa, and in highly populated areas such as India. HIV-1B, accounting for approximately 10% of the HIV-1 infections, predominates in the Americas and in Western Europe.[1] HIV-1B also predominates in Brazil, but subtypes F, C and associated recombinant forms co-circulate with expressive frequency (Fig. 1).[2–5] However in the Southern region, a distinct profile is observed. Currently, depending on the state, 27–79% of HIV infections are caused by HIV-1C, 23–45% by HIV-1B, 3–29% by CRF31_BC and other BC recombinants, and HIV-1F and its recombinants account for up to 10% of infections.[6–16]

Recent studies suggest that the HIV-1 epidemics in Brazil has initiated with multiple introductions of HIV-1B during the 60s or 70s[17,18] in the largest cities of Southeast, and then disseminated throughout the country. HIV-1F and C had a monophyletic entry in the country, the former in the Southeast and the latter in the South, approximately 10–15 years after HIV-1B.[18,19] HIV-1F appears to have originated from Central Africa,[18] whereas HIV-1C was likely introduced from East Africa.[20–22]

The association of heterosexual transmission with non-B HIV subtypes, and that of men who have sex with men and subtype B has been well characterized. However, such associations were

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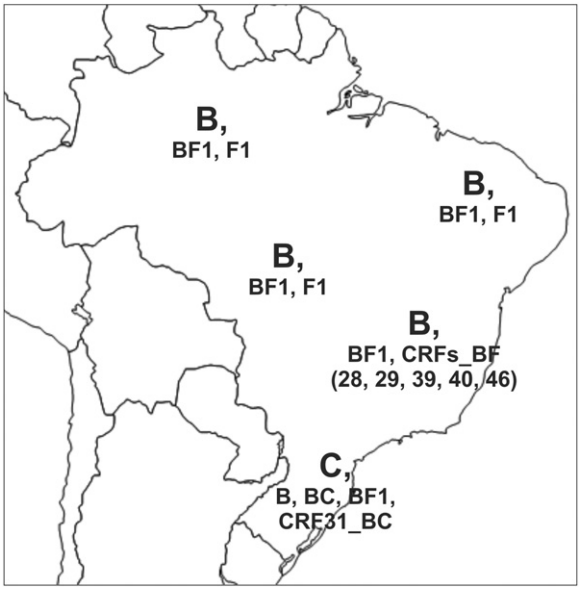

Fig. 1. Map of Brazil depicting the prevalence of the major HIV-1 subtypes and recombinant forms (CRFs and URFs) in each of the five macro-regions of the country (North, Northeast, Center-West, Southeast and South). Prevalence is proportional to the size of the font in the map. Only HIV-1 forms with significant prevalence (above 5%) are shown.

rather explained by ethnicity and locale of contamination rather than by HIV-1 subtypes *per se*.[23,24] In Southern Brazil, where HIV-1B and C co-circulate in a homogeneous population, previous studies by our group[11,25] and by others[26] have suggested an association of HIV-1C with heterosexual transmission. More recently, a study conducted in Florianópolis, Santa Catarina, proved such association with confidence,[15] but no corroboration has been so far shown in Rio Grande do Sul, where HIV-1C likely entered Brazil and where the highest HIV-1C prevalence occurs.

2. Objectives

The present study aimed at evaluating the prevalence of HIV-1 subtypes and recombinant forms in patients with recent diagnosis for HIV infection followed up at a HIV/AIDS reference center in the city of Rio Grande, in the southernmost region of Brazil. We also studied the association of the HIV subtypes found with demographic, behavioral, clinical and laboratorial characteristics retrieved at the moment of inclusion in the study.

3. Study design

Study subjects and sample collection: Individuals of age 18 years or older diagnosed for HIV infection between January 2005 and December 2008 were invited to participate in the study. Subjects were enrolled after signing an informed consent. The study has been approved by the Ethics Committee of the Universidade Federal de Rio Grande University Hospital under the protocol no. 63/2008. Samples were collected for initial laboratory tests ($CD4^+$ T-cell counts and HIV viral load measurements) and for HIV-1 subtype determination using plasma samples obtained at HIV diagnosis. An aliquot of plasma was preserved at −80 °C for further molecular analyses.

Viral RNA extraction, cDNA synthesis and PCR: Viral RNA extraction was done with the QIAamp Viral RNA kit (QIAGEN, Chatsworth, USA), according to the manufacturer's instructions and as previously described.[13] Nested PCR were conducted to amplify the virus genomic *pol* regions of protease (PR), and reverse transcriptase polymerase (RT), connection (CN) and RNase H (RH) domains. Primers and PCR conditions used were those described previously for PR and RT,[13] For CN and RH, a fragment of 962 bp containing both regions was amplified with a nested PCR using the primers ConX1 (5′tggatgggttatgaact3′) and INT_R (5′cagtctacttgtccatgcatggcttc3′) in the first round; and ConX2 (5′atacagaagttagtgggaaaa3′) and Out3R (5′cattgctctccaattactgtgatatttctcatg3′) in the second round. When the amplification was not successful, the four genomic regions were amplified separately.

Sequence alignments and phylogenetic analyses: PCR products were sequenced in a 3130XL Genetic Analyzer (Life Technologies, Carlsbad, USA), and sequences were assembled and edited using the software SeqMan of DNAStar (DNAStar, Madison, USA) in a PC/Windows. Edited sequenced were aligned with reference HIV sequences retrieved from the Los Alamos HIV Database (http://hiv-web.lanl.gov/) comprising all HIV-1 subtypes. Alignments were made in ClustalW,[27] and further used in phylogenetic inferences using MEGA 4.1[28] for HIV-1 subtype assignment. The neighbor-joining distance method was used, with Kimura 2-parameter correction. Phylogenetic cluster confidence was estimated by bootstrap (1000 replications). Sequences with discordant phylogenetic classification in different genomic fragments were subjected to recombination analysis by *bootscanning*, available in Simplot v.3.5.1. Sequences were compared to reference sequences of the most similar HIV-1 subtypes.

Statistical analyses: Demographical and clinical data of patients were entered in Excel and then imported into Stata v.9.2. Cohort descriptive data were studied with averages, standard deviations, medians and ranges for continuous variables, and absolute and relative frequencies for categorical variables. We also calculated absolute and relative frequencies of different HIV-1 subtypes with their respective 95% confidence intervals. A univariate analysis was conducted to evaluate the association of demographic, behavioral, clinical and laboratorial variables with HIV-1 subtypes, using Chi-square or Fisher's exact tests. For categorical ordinal variables, linear trends were evaluated. For continuous variables, the Student's *t*-test was employed, and for those with non-normal distribution, the Kruskal–Wallis non-parametric test was used. Differences were considered significant when p values ≤ 0.05 were achieved.

4. Results

Two hundred forty-five samples from subjects with recent HIV-1 diagnosis between 2005 and 2008 were successfully characterized. The association between molecular data with demographical and clinical data was conducted for 233 (95.1%) cases. The year distribution of HIV diagnoses was: 44 (18.9%) in 2005, 66 (28.3%) in 2006, 70 (30%) in 2007 and 53 (22.8%) in 2008. One hundred twenty-nine subjects (55.4%) were male, with a male-to-female (M:F) ratio of 1.24:1. Additional demographical and clinical data of the patients studied and their relation with gender are shown in Table 1. A difference in the median CD4+ T-cell counts between males and females was initially observed. However, when the 22 pregnant women (9.4% of the total sample and 21.1% of females) present in the cohort were excluded from the analysis, the median CD4+ T-cell counts in females decreased to 324 cells/mm^3, and the significance of the difference from males was lost ($p = 0.1$; data not shown).

Of the 245 HIV-1 isolates studied, we obtained sequence data of PR for 141 (57.6%) and of RT polymerase domain for 87 (35.5%). For the C-terminal RT domains, we analyzed 164 (66.9%) samples for CN, and 168 (68.6%) for RH. As seen, the entire *pol* gene was not obtained for all viruses, but the fragments analyzed were

Table 1
Demographic, behavioral, clinical and laboratorial characteristics of the studied population, and their distribution according to gender, HU/FURG, Rio Grande, 2005–2008 (n = 233).

Characteristic	Total 233 n (%)	Female 104 n (%)	Male 129 n (%)	p
Age group				
18–24	44 (18.9)	30 (28.9)	14 (10.8)	0.006
25–34	73 (31.3)	28 (26.9)	45 (34.9)	
35–49	88 (37.8)	36 (34.6)	52 (40.3)	
≥50	28 (12.0)	10 (9.6)	18 (14.0)	
Transmission route (n = 212)[*]				
Heterosexual	162 (76.4)	92 (95.9)	70 (60.3)	0.001
Homosexual	25 (11.8)	0	25 (21.6)	
IDU	24 (11.3)	3 (3.1)	21 (18.1)	
Other	1 (0.5)	1 (1.0)	0	
HIV$^+$ partner	52 (22.4)	30 (28.9)	22 (17.1)	0.032
Partner on ARV (n = 218)[*]	10 (4.3)	7 (7.5)	3 (2.4)	0.079
AIDS diagnosis[**]	109 (46.8)	39 (37.5)	70 (54.3)	0.01
CDC clinical category				
A	127 (54.5)	68 (65.4)	59 (45.7)	0.008
B	27 (11.6)	11 (10.6)	16 (12.4)	
C	79 (33.9)	25 (24.0)	54 (41.9)	
CDC immunological category[***]				
1	62 (26.6)	35 (33.6)	27 (20.9)	0.051
2	83 (35.6)	37 (35.6)	46 (35.7)	
3	88 (37.8)	32 (30.8)	56 (43.4)	
HCV$^+$ (n = 225)[*,a]	20 (8.9)	7 (6.9)	13 (10.6)	0.3
Tuberculosis (n = 231)[*,a]	34 (14.7)	8 (7.7)	26 (20.5)	0.006
Death in the 1st year	26 (11.2)	11 (10.6)	15 (11.6)	0.8
	Median (IQR)			
CD4$^+$ T-cell counts	317 (104–520)	367 (138–578)	247 (71–465)	0.01[****]
Plasma HIV viral load (cp/mL)	30,693 (8638–131,007)	18,920 (4666–105,194)	42,464 (11,777–141,990)	0.007[****]

IDU, intravenous drug user.
[a] At the date of HIV diagnosis.
[*] Number of cases for which the variable was available.
[**] CDC 1993 criteria.
[***] CDC 1993 criteria – 1, ≥500/mm^3; 2, between 499 and 200/mm^3; 3, ≤199/mm^3.
[****] Kruskal–Wallis test.

Table 2
HIV-1 subtype distribution according to demographic and behavioral characteristics studied, HU/FURG, Rio Grande, 2005–2008 (n = 233).

Characteristic	Subtype B 49 n (%)	Subtype C 131 n (%)	Subtype F1 13 n (%)	Recombinants 40 n (%)	p
Gender					
Female	19 (38.8)	63 (48.1)	5 (38.5)	17 (42.5)	0.6
Male	30 (61.2)	68 (51.9)	8 (61.5)	23 (57.5)	
Age group					
18–24	8 (16.4)	26 (19.8)	1 (7.7)	9 (22.5)	0.1
25–34	10 (20.4)	48 (36.7)	7 (53.8)	8 (20.0)	
35–49	25 (51.0)	43 (32.8)	3 (23.1)	17 (42.5)	
≥50	6 (12.2)	14 (10.7)	2 (15.4)	6 (15.0)	
Place of residence					
Rio Grande/Pelotas	43 (87.8)	120 (91.6)	10 (76.9)	36 (90.0)	0.3
Uruguay border	6 (12.2)	11 (8.4)	3 (23.1)	4 (10.0)	
Transmission route (n = 212)[*]					
Heterosexual	27 (61.4)	100 (83.3)	9 (69.2)	26 (74.3)	0.001
Homosexual	14 (31.8)	5 (4.2)	2 (15.4)	4 (11.4)	
IDU	3 (6.8)	15 (12.5)	2 (15.4)	5 (14.3)	
HIV$^+$ partner	11 (22.4)	31 (23.7)	3 (23.1)	7 (17.5)	0.8
AIDS diagnosis[**]	23 (46.9)	57 (43.5)	6 (46.2)	23 (57.5)	0.4
CDC clinical category					
A	25 (51.0)	75 (57.2)	9 (70.0)	18 (45.0)	0.4
B	9 (18.4)	12 (9.2)	1 (7.7)	5 (12.5)	
C	15 (30.6)	44 (33.6)	3 (2.3)	17 (42.5)	
CDC immunological category[***]					
1	12 (24.5)	38 (29.0)	4 (30.8)	8 (20.0)	0.7
2	17 (34.7)	49 (37.4)	4 (30.8)	13 (32.5)	
3	20 (40.8)	44 (33.6)	5 (38.4)	19 (47.5)	
Death in the 1st year	8 (16.3)	12 (9.2)	0	6 (15.0)	0.2
	Median (IQR)				
CD4$^+$ T-cell counts	263 (64–465)	350 (136–540)	326 (72–635)	235 (58–441)	0.1[****]
Plasma HIV viral load (cp/mL)	48,114 (10,634–113,755)	24,141 (6183–127,721)	45,508 (19,716–101,710)	30,280 (12,081–157,391)	0.5[****]

IDU, intravenous drug user.
[*] Number of cases for which the variable was available.
[**] CDC 1993 criteria.
[***] CDC 1993 criteria – 1, ≥500/mm^3; 2, between 499 and 200/mm^3; 3, ≤199/mm^3.
[****] Kruskal–Wallis test.

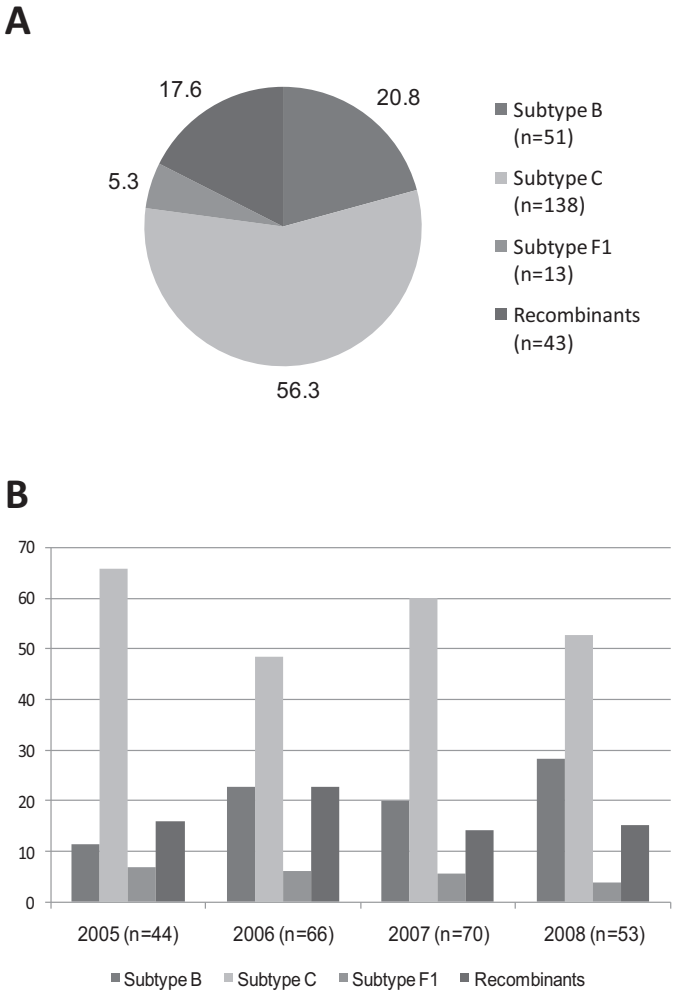

Fig. 2. (A) Prevalence of HIV-1 subtypes in the casuistic analyzed, University Hospital, Universidade Federal de Rio Grande, 2005–2008 ($n = 245$). (B) HIV-1 subtype distribution according to year of HIV diagnosis, University Hospital, Universidade Federal de Rio Grande, 2005–2008 ($n = 233$).

sufficient to classify all of them phylogenetically. The conjoint analysis of these genomic fragments showed that 51 (20.8%; 95% CI 15.7–25.9) were subtype B viruses, 138 (56.3%; 95% CI 50.1–62.6) were subtype C, 13 (5.3%; 95% CI 2.5–8.1) were subtype F and 43 (17.6%; 95% CI 12.7–22.3) were recombinant forms (Fig. 2A). The prevalence of HIV-1 subtypes remained constant over the period studied, predominated by subtype C (Fig. 2B). In the 12 samples for which no clinical data was studied (see above), the relative frequencies of HIV-1 subtypes were similar to the casuistic analyzed (not shown).

The phylogenetic analysis of the recombinant forms found evidenced one CRF31_BC and one CRF28/29_BF. For the latter, the fragment analyzed did not allow the discrimination of the two CRFs (28_BF or 29_BF), as these two have the same ancestral origin for the region analyzed. The remaining 41 recombinant forms were all unique (URFs): 26 B/C, 7 B/F, one B/F/C and 7 CRF12_BF-derived recombinants (data not shown).

When demographic and behavioral characteristics were compared between viral subtypes, a significant association was observed between the reported route of HIV acquisition and infecting subtype. Heterosexual transmission was more prevalent among subtype C-infected subjects (61.7%), whereas MSM predominated among subtype B-infected subjects (56%) ($p = 0.001$). The remaining demographic, behavioral and clinical characteristics studied did not show differences between infecting subtypes (Table 2). With respect to geographic origin, subjects from the border with Uruguay also showed higher prevalence of HIV-1C (Table 2), and none of the 7 isolates harboring CRF12_BF-derived genomic fragments belonged to patients originated from that locale.

Since HIV-1C represented the majority of strains in our casuistic, data were grouped for subtype C and non-subtype C-infected subjects. Comparison of these groups revealed that the association between HIV-1C infection and heterosexual transmission remained significant ($p = 0.001$). In addition, a difference in $CD4^+$ T-cell counts at study entry was found between the two groups, with subjects in the subtype C group presenting a median of 350 cells/mm^3 (IQR 16-540) and the non-subtype C a median of 246 (IQR 64-489) ($p = 0.02$, Kruskal–Wallis test). We have also adjusted the distribution of HIV-1 subtypes (C or non-C) for gender and age categories. None of the correlations were statistically significant, with the exception of the age group between 18 and 24 years, in which subtype C was correlated with women and non-C with men.

5. Discussion

The recently diagnosed HIV-1^+ subjects were composed of 55.4% of men and 44.6% of women and had an average age of 36 years. The M:F ratio of 1.24:1 and the predominance of heterosexual transmission, responsible for 76.4% of cases, are compatible with the current epidemiological scenario of HIV infection in Brazil (http://www.aids.gov.br).

An important observation corroborated by this study is that despite the success of the Brazilian AIDS Program in AIDS prevention and treatment, HIV diagnosis is late in a significant part of the infected subjects. In this study, 37.5% of the subjects presented levels of $CD4^+$ T-cell counts below 200 cells/mm^3, and 54.9% of them had counts below 350 cells/mm^3, evidencing a significant proportion of lately diagnosed subjects according to the Brazilian guidelines.

The molecular epidemiology results of this study corroborate previous reports from southern Brazil, showing the predominance of HIV-1C, followed by HIV-1B, and lastly by other forms.[6,7,11,12,14,15,25,29] CRF31_BC, described by our group in 2006,[29] was rarely found here. Among the 27 B/C recombinant viruses observed, only one harbored recombination breakpoints compatible with CRF31_BC. This is consistent with reports showing that the growth rate of CRF31_BC is lower than that of HIV-1C.[11,18] Moreover, despite the fact that the prevalence of CRF31_BC strains increased from 9% in 2002 to 25% in 2004 in Porto Alegre,[11] in Rio Grande and in Itajaí, SC, cities with similar socio-demographic and cultural characteristics, the prevalence of this CRF remained below 4% in the same period.[6,11]

We were not able to PCR amplify the entire *pol* gene from all viruses. Since the cohort analyzed here was of consecutive enrollment, irrespective of HIV VL at the time of collection, some subjects had low (<1000 copies) VL, which could at least in part explain the failures of the amplifications observed. Moreover, the high genetic diversity observed, with multiple subtypes and recombinants, could also impair primer annealing and consequently PCR efficiency. However, the congruence between the subtype distribution seen herein and that previously reported in southern Brazil validates our results.

An important proportion of HIV^+ subjects residing in the bordering cities to Uruguay (45%) were infected with HIV-1C. This is the first report showing such a relevant circulation of this subtype in the area. Previous research conducted in Argentina, Uruguay and Paraguay showed only marginal circulation of HIV-1C, with prevalence rates below 1%.[30–33] Conversely, seven different viral isolates harboring CRF12_BF-derived fragments were found inwards of the

border to Uruguay, an observation not previously reported in the region.[10] The identification of this CRF in inland Brazil shows the changing dynamics of the circulation of HIV-1 strains, which warrants periodic molecular surveillance.

The lack of significant associations between infecting HIV-1 subtypes and gender, age groups, locale of residence, HIV$^+$ partners, and clinical characteristics in this casuistic is relevant and shows the homogeneity of the cohort studied. The association between the transmission route and viral subtypes is therefore paramount. Heterosexual transmission was more prevalent in HIV-1C, whereas the homosexual route was more frequent in HIV-1B. The significance was maintained when we grouped all non-C viruses compared to HIV-1C, the most frequent in our casuistic. Despite several previous studies have suggested that association,[11,15,25,26] this is the first showing it with statistical robustness in the state of Rio Grande do Sul, where HIV-1C likely entered Brazil.

HIV-1C-infected patients showed higher CD4$^+$ T-cell counts compared to other (non-C) variants. This observation could be explained by the more recent epidemic of HIV-1C in Brazil.[20] This subtype, however, rapidly expanded in countries where it has been introduced.[34–36] An alternative explanation for this phenomenon may rely on viral fitness. CCR5-tropic HIV-1C has been shown to harbor equivalent transmission fitness to other HIV-1 group M subtypes, but its virulence was estimated some 100 times lower.[37,38] The lower within-host pathogenicity displayed by HIV-1C might be linked to a scenario where the host remains disease-free for a longer time, yet with similar transmission ability, leading to increased proportion over time in the population. Longitudinal studies in places with homogeneous populations infected by different HIV-1 subtypes are required to elucidate this hypothesis.

In summary, this study showed a robust association between HIV-1C and heterosexual transmission within a population of homogeneous demographic and ethnic characteristics. Southern Brazil is an ideal locale for those studies, as relevant frequencies of HIV-1C and B are seen. This area displays unique features that allow the exclusion of confounding factors seen in most studies that evaluate HIV-1 subtype influence on disease progression.

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Competing interest

None declared.

Ethical approval

Ethical Approval was given by the Ethics Committee of the Universidade Federal do Rio Grande University Hospital (CEPAS) under the protocol # 63/2008.

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